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OBSERVATIONS ON MILDEW SUSCEPTIBILITY OF PAINTED SURFACES IN TROPICAL CHAMBER EXPOSURE

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Painted specimens of varying fungal susceptibilities were evaluated in tropical chamber exposure. Differences in results were attributed to differences in environmental conditions, substrate, fungal inoculum and nutrient salts or soil. It was concluded that a cycled walk-in tropical chamber used for microbial screening of various Army materiel supported satisfactory fungal growth on painted surfaces.

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PREFACE

Standard test procedures for laboratory evaluation of the fungal susceptibility of paint coatings require differing specialized test cabinets, organisms, and substrates. Experience has shown that our walk-in tropical chambers are suitable for evaluating the fungal susceptibility of a variety of Army materiel without need for specialized pretreatment or environmental conditions. This report describes the results from four tropical chamber studies performed on mildew susceptible and mildew resistant paint coatings in an attempt to determine the role of mixed fungal inoculum, substrate, nutrient salts, and environmental conditions on the mildew susceptibility of the coatings. The data were accumulated over several years under Work Unit CH001 93223415001 through 23223415001.

These studies were made possible by personnel from other laboratories who assisted by supplying us with paints and painted panels for our evaluation. Dr. Milton Goll, Cosan Chemical Corporation, Clifton, NJ, supplied vinyl acrylic paints, both with and without phenyl mercuric acetate. Mr. W. R. Springle, Paint Research Association, Teddington, UK, provided International Biodeterioration Research Group (IBRG) test organisms, and IBRG prepared wood and plaster panels. Ms. Sarah Rosen, US Army Electronics Research and Development Command (ERADCOM), Ft. Monmouth, NJ, supplied all paints conforming to military specifications and all metal panels treated with military paints.

Other personnel, from the US Army Natick Research & Development Center, contributed to this undertaking. Ms. Cynthia A. Harrington and Ms. Kyle A. Wallace participated in the early experimentation. Dr. David L. Kaplan provided guidance for preparation of the manuscript.

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INTRODUCTION

This report contains results from four experiments on painted surfaces incubated in large multipurpose tropical chambers contaminated with organisms commonly found in nature.[1] These experiments attempt to establish the usefulness of the tropical chamber for the evaluation of mildew susceptibility of painted surfaces and to establish the conditions necessary for satisfactory growth in a tropical chamber. No attempt was made to isolate and identify species populating the inoculated and uninoculated test films after tropical exposure, but *Aureobasidium pullulans*, the organism most commonly associated with paint mildew, was included in two fungal spore sprays and has been identified as a habitant of our chamber.[1]

Over the years our tropical chambers have yielded satisfactory results for a variety of Army materiel exposed under Method 508.1[2] conditions of temperature and humidity but without application of fungal sprays or special pretreatment. Our interest in evaluating the effect of test variables was only to determine whether there are any special requirements for supporting growth on painted surfaces exposed in tropical chambers. Certainly, the literature on mildew susceptibility of paint films is replete with studies on effect of test variables.

Extensive work on the mold resistance of paint films has been performed under the auspices of the Paints Working Group of the International Biodeterioration Research Group (IBRG) by ten participating international laboratories.[3] One of the many important observations made during these studies was that panels of glass, aluminum, or wood exposed in special humidity cabinets[3,4] resulted in data comparable to that obtained from glass test tubes -- a test method originally devised by Hendey[5] and modified by Barry *et. al*.[6] If so, paint films could then be tested on substrates used in actual practice. This finding is relevant to both civilian and military paint applications. Consequently, studies were undertaken in our laboratory to determine whether satisfactory mildew susceptibility data could be obtained for flat painted surfaces exposed in our multipurpose, walk-in tropical chambers.

MATERIALS AND METHODS

Sample Preparation

Test samples for tropical chamber exposure included both paints and painted surfaces. Paints included defined exterior white vinyl acrylic paints with and without 1.8 kg/m^3 phenylmercuric acetate (PMA) from a commercial supplier (Table 1) and three forest green paints prepared to meet military specifications (Table 2). The three paints were an alkyd enamel,[7] a modified alkyd[8] and an aliphatic polyurethane coating.[9] These were evaluated by dipping 153 x 19 x 1.8 mm polished gumwood and hardwood tongue depressors into each paint and then allowing the coated surfaces to drip-dry and harden overnight.

TABLE 1. Composition (kg/m3) of Experimental Exterior Vinyl Acrylic Paint

Raw Materials	Control Untreated	Fungicide-Treated
Water	119.8	119.8
Propylene glycol	28.8	28.8
Hydroxyethyl Cellulose (thickener)	167.7	167.7
Phenylmercuric acetate (PMA)	0	1.8
Sodium salt of a carboxylic acid (dispersant)	14.4	14.4
Potassium tripolyphosphate	4.8	4.8
Mineral oil based defoamer	1.2	1.2
Titanium dioxide	299.5	299.5
Calcium carbonate	59.9	59.9
Magnesium silicate (extender)	59.9	59.9
Ester alcohol (solvent)	9.6	9.6
Water	67.1	67.1
Mineral oil based defoamer	2.4	2.4
Vinyl acrylic latex	431.3	431.3
Dioctyl sodium sulfosuccinate (surfactant)	2.4	2.4

TABLE 2. Composition of Forest Green Military Specification Paints

Paint	Pigment	Vehicle
MIL-E-52835A Enamel, Modified Alkyd Camouflage, Lusterless	Acid insoluble green composed of cobalt, zinc, and chromium oxides, and/or chromate yellow, molybdate orange, carbazole dioxazine violet, yellow iron oxide, red iron oxide.	Pure short oil length phthalic alkyd resin baking type, modified with not less than 20% butylated melamine formaldehyde resins of urea formaldehyde or blends of urea/melamine + modifiers, stabilizers, wetting and suspension agents.
MIL-E-52798A Enamel, Alkyd Camouflage	Same as MIL-E-52835A	Drying oil phthalic alkyd resin in mineral spirits. Driers and Volatile Solvents: Alkyd resin solution Color Phthalic anhydride Drying oil acids Unsaponifiable matter
MIL-C-46168A (MR) Coating, Aliphatic Polyurethane, Chemical Agent Resistant	Same as MIL-E-52835A	Non volatile: Dicarboxylic Acid Polyols Aliphatic polyisocyanate Volatile: Aromatic compounds Ethyl benzene and toluene Solvents, olefinic or cyclo-olefinic Ethylene glycol monethyl ether acetate

Painted surfaces received for evaluation were prepared by either the Electronics Research and Development Command (ERADCOM) or the Paints Working Group of IBRG. ERADCOM specimens were 102 x 305 x 1.1 mm steel panels coated with the three military specification paints specified above. IBRG specimens were painted flat panels, 100 x 75 x 10 mm, of either wood or plaster. Wood panels were select specimens of straight-grained sapwood or Scots pine (*Pinus sylvestris*). Plaster panels were formulated from a 3:2 hemi-hydrate plaster to distilled water mix. All IBRG panels were conditioned at 20 $\pm$ 2°C and 65 $\pm$ 5% RH prior to painting. IBRG panels were painted with linseed oil, alkyd gloss, alkyd semi-gloss, polyvinylacetate (PVA) emulsion, alkyd modified acrylic, alkyd modified acrylic plus 1% tetra methyl thiuram disulphide (TMTD), and acrylic emulsion. All seven paints were evaluated on wood panels, but only alkyd gloss and alkyd modified acrylic, both with and without TMTD, were evaluated on plaster. Two coats were applied to each panel. Twenty-four hours was allowed as drying time between coats of oil-based paints and one hour between coats of emulsion paints.

Two of each set of four IBRG wood panels were weathered one side only for 242 hours with spray in a carbon arc Weather-Ometer.[10]

All painted surfaces were exposed in one or both of the following tropical environments:

1. An uncycled chamber, 3.3 x 3.3 x 2.6 m, operated continually at 30°C $\pm$ 1°C and 95% RH.

2. A cycled chamber, 6.9 x 7.5 x 3.6 m, operated continually for 20 hours at 30°C $\pm$ 1°C and 95% RH followed by 4 hours at 25°C $\pm$ 1°C and 100% RH. Cycle conditions were in accordance with Method 508.1.[2]

Susceptibility Tests

Prior to incubation, painted surfaces were sprayed with fungal spores and/or nutrient salts or left unsprayed. Spore mixtures and nutrient salts were prepared in accordance with the following procedures:

1. ASTM D3273-76.[4]
2. IBRG test procedure.[6]
3. Method 508.1 Fungus.[2]

IBRG nutrient soil was prepared in our laboratory from a nutrient salts/starch paste containing trace elements. Nutrient salts/starch paste was prepared from 17.0 g potato starch mixed with 20 mL distilled water to form a thin slurry. The slurry was poured into 120 mL boiling distilled water to form a gel. To the gel was added nutrient salts in 60 mL distilled water and 1 mL trace element solution (Table 3).

TABLE 3. Composition of IBRG Nutrient Soil

<u>Nutrient salts (g/60 mL)</u>		
Calcium phosphate, monobasic	[$CaH_4(PO_4)_2.H_2O$]	0.44 g
Calcium phosphate, tribasic	[$Ca_3(PO_4)_2$]	0.90 g
Calcium carbonate	[$CaCO_3$]	0.20 g
Potassium sulfate	[K_2SO_4]	0.11 g
Ammonium sulfate	[$(NH_4)_2SO_4$]	0.51 g
Ammonium nitrate	[NH_4NO_3]	1.37 g
<u>Trace elements (g/100 mL)</u>		
Ferric chloride	[$FeCl_3 \cdot 5H_2O$]	1.46 g
Cupric sulfate	[$CuSO_4 \cdot 5H_2O$]	1.18 g
Zinc sulfate	[$ZnSO_4 \cdot 7H_2O$]	1.32 g
Ammonium molybdate	[$(NH_4)_6Mo_7O_{24}$]	0.01 g
Manganous sulfate	[$MnSO_4 \cdot 4H_2O$]	0.20 g

The nutrient salts/starch paste/trace element mixture was beaten and water added until mixture weight was 249 g. Twenty grams of the mixture was added to 20 g of 75 μm (200 mesh) glass powder and mixed. The mixture was spread onto Whatman IPS silicone-treated phase separating paper and dried for 3 hours at 100°C. After drying, the mixture was passed through a 180 μm (80 mesh) sieve and stored in a sterile container.

Method 508.1 (ASTM G 21-70) nutrient salts medium was prepared from the salts listed in Table 4. The salts were dissolved in one liter of distilled water, adjusted to a pH of 6.0 to 6.5 and autoclaved at 121°C for 20 minutes at 1.03×10^5 Pa (15 psi).

TABLE 4. Composition of Method 508.1 (ASTM G21-70) Nutrient Salts (g/L)

potassium dihydrogen orthophosphate (KH_2PO_4)	0.7 g
potassium monohydrogen orthophosphate (K_2HPO_4)	0.7 g
magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.7 g
ammonium nitrate (NH_4NO_3)	1.0 g
sodium chloride (NaCl)	0.005 g
ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	0.002 g
zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.002 g
manganous sulfate ($MnSO_4 \cdot H_2O$)	0.001 g

IBRG fungal spore suspension consisted of the test organisms listed in Table 5. Ten mL of distilled water containing 0.05 g/L Tween 80 were added to each of the cultures. The cultures were scraped, and the suspensions were filtered through sterile glass wool and combined in a sterile flask. A concentration of at least 10^4 spores/mL was verified with a counting chamber.

ASTM fungal spore suspension consisted of the test organisms listed in Table 5. Five mL of distilled water containing 0.05 g/L Tween 80 were added to each of the cultures. The cultures were scraped, and the suspensions were filtered through sterile glass wool, combined in a sterile flask, and diluted with surfactant-sterile water mixture to a total volume of 95 - 100 mL.

TABLE 5. Test Organisms

Test method		
ASTM D-3273-76	QM 458	*Aspergillus niger*
	QM 1226	*Penicillium citrinum*
	QM 3090	*Aureobasidium pullulans*
IBRG test procedure	IMI 17,454	*Aspergillus niger*
	IMI 45,533	*Aureobasidium pullulans*
	IMI 45,554	*Aspergillus versicolor*
	IMI 49,948ii	*Phoma violacea*
	IMI 79,906	*Ulocladium atrum*
	IMI 82,021	*Stachybotrys atra*
	IMI 178,517	*Cladosporium cladosporioides*
	IMI 178,519	*Penicillium purpurogenum*
Method 508.1	QM 386	*Aspergillus niger*
	QM 380	*Aspergillus flavus*
	QM 432	*Aspergillus versicolor*
	QM 474	*Penicillium funiculosum*
	QM 459	*Chaetomium globosum*

Method 508.1 fungal spore suspension consisted of the test organisms listed in Table 5. Ten mL of distilled water containing 0.05 g/L Tween 80 were added to each of the cultures. The cultures were scraped and the suspensions were filtered through sterile glass wool in separate flasks, with the exception of QM 459 *Chaetomium globosum*. The spore charge of *C. globosum* was poured into a sterile 125 mL screw cap flask containing approximately 50 to 75 glass beads. The flask was shaken to liberate the spores from the fruiting bodies. The spore charge was then filtered through glass wool. Each spore charge was then diluted to 50 mL with sterile distilled water and centrifuged. The supernatant was discarded, the residue resuspended in 50 mL sterile distilled water and centrifuged. The spores were washed in this manner three times. The final washed residue was diluted with sterile nutrient salts solution so that the resultant spore suspension was 1,000,000 ± 200,000 spores/mL as determined with a counting chamber. Equal volumes of each of the resultant spore suspensions were blended to obtain the final mixed spore suspension.[2]

IBRG panels were inoculated only on the front weathered or unweathered surface (back surfaces were not weathered or inoculated). One inoculated panel of each type was coated with nutrient soil.

The painted metal panels from ERADCOM were the only painted surfaces to be cleaned, and they were cleaned with 70% ethanol 72 hours prior to inoculation. After inoculation, painted surfaces were incubated in walk-in tropical chambers operated at either cycled or uncycled conditions. Mold growth was rated at weekly intervals for at least four weeks based on the rating scale used by Barry et al.[6]

RESULTS

Experiment #1. Effect of cycled and uncycled environmental conditions on mildew susceptible and resistant vinyl acrylic paints on wood.

Gumwood depressors were coated with defined vinyl acrylic paints, and after drying were sprayed with one of the following: IBRG fungal spore mixture, ASTM fungal spore mixture, Method 508 nutrient salts, or left unsprayed. Specimens were then exposed in either the cycled or uncycled tropical chamber. Growth ratings from these paints are listed in Table 6. Results from the uncycled chamber indicate that untreated paints, as expected, supported more growth than paint containing PMA. All three untreated paint sets exposed in the uncycled chamber had comparable early growth ratings, but later ratings showed more divergence, as the set sprayed with ASTM fungal inoculum supported more growth than the other two sets. For PMA-treated specimens, data from uninoculated and inoculated sets were more closely alike than comparable data from untreated sets, particularly for sets exposed in the cycled chamber. Treated sets exposed in the cycled chamber supported only trace growth with no increase in growth during the 28-day exposure. Data from the untreated paint sets indicate that conditions in the cycled chamber were more conducive for growth than conditions in the uncycled chamber. All untreated paint sets eventually achieved more growth in this chamber. Spraying with either ASTM or IBRG fungal spores gave the added advantage, relative to uninoculated specimens, of more growth and more uniform surface response at the beginning of the exposure.

Cycled chamber exposure was repeated for untreated paint sets, but with the inclusion of ASTM nutrient salts. Again, more growth was obtained through use of fungal spore sprays. However, since the uninoculated specimens supported more growth at the beginning of the exposure than in the previous trial, differences between inoculated and uninoculated sets were not as large as differences seen in the earlier cycled exposure. Differences between the unsprayed set and the set sprayed with nutrient salts were even smaller.

TABLE 6. Growth Ratings[a] for Gumwood Depressors Coated with Vinyl Acrylic Paints

	Tropical Chamber Exposure in Days			
Uncycled Chamber	7	14	21	28
paint, untr, no spray	3.1 (1.1)[b]	3.1 (1.1)	3.1 (1.1)	3.5 (1.2)
IBRG fungal inoculum	2.6 (1.1)	2.6 (1.1)	2.6 (1.1)	2.6 (1.1)
ASTM fungal inoculum	3.0 (1.1)	3.1 (1.1)	3.8 (1.2)	4.4 (0.5)
paint w/PMA, no spray	1.2 (0.5)	1.2 (0.5)	1.2 (0.5)	1.2 (0.5)
IBRG fungal inoculum	1.8 (1.5)	2.0 (1.4)	2.1 (1.5)	2.2 (1.6)
ASTM fungal inoculum	1.2 (0.5)	1.8 (1.4)	1.8 (1.4)	1.8 (1.4)
Cycled Chamber				
paint, untr, no spray	2.5 (1.6)	3.8 (1.4)	3.8 (1.4)	4.8 (0.5)
IBRG fungal inoculum	4.5 (0.8)	4.6 (0.5)	4.4 (0.5)	5.0 (0)
ASTM fungal inoculum	4.5 (1.1)	4.6 (0.7)	4.6 (0.7)	4.9 (0.4)
paint, w/PMA, no spray	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)
IBRG fungal inoculum	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)
ASTM fungal inoculum	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)
Cycled Chamber (repeat)				
paint, untr, no spray	3.2 (0.9)	4.0 (1.1)	4.6 (0.5)	4.8 (0.5)
ASTM salts	3.4 (0.7)	4.4 (0.5)	4.4 (0.5)	4.6 (0.5)
ASTM fungal inoculum	4.0 (0)	4.8 (0.5)	5.0 (0)	5.0 (0)
IBRG fungal inoculum	4.8 (0.5)	5.0 (0)	5.0 (0)	5.0 (0)

[a]Ratings represent the average of four specimens.

Rating scale as follows:

0 = no growth
1 = trace growth
2 = 1-10% coverage of test surface
3 = 10-30% coverage
4 = 30-70% coverage
5 = 70-100% coverage

[b]Standard Deviation

Experiment #2. Effect of mixed fungal inoculum with and without nutrient soil on the mildew susceptibility of oil- and water-based paints on wood and plaster.

In a second experiment, growth ratings were obtained from wood and plaster panels prepared under the IBRG collaborative program for paints. After weathering, specimens were inoculated with IBRG fungal spore mixture or IBRG fungal spore mixture plus nutrient soil containing starch, or left unsprayed. Weathered and unweathered specimens were exposed only in the cycled chamber because of better environmental conditions for growth.

Table 7 contains a compilation of the data from the second experiment. With wood as substrate, spraying with IBRG fungal spore mixture resulted in more growth at the beginning of the exposure, except for coating H (acrylic emulsion) on wood. The combination of IBRG fungal spore inoculum plus nutrient soil resulted in maximum growth at one week of exposure for each coating on wood or plaster, whether unweathered or weathered. Again, as seen in the earlier work with vinyl acrylic paint on wood, differences within sets narrowed at the end of the 28-day exposure as growth on unsprayed specimens increased.

Ratings for laboratory weathered coatings on wood were compared to the same five types of painted wood before weathering (Table 8). On average, weathered painted wood was slightly more susceptible than unweathered painted wood, but if standard deviations are considered, both sets of data are about the same. As with unweathered painted wood specimens, the growth ratings of weathered painted specimens were increased by prior inoculation with either IBRG fungal spore mixture or IBRG fungal spore mixture plus nutrient soil.

Fungal growth ratings of the painted plaster panels increased with the addition of IBRG fungal spore inoculum, but the most growth was obtained by inclusion of the nutrient soil. Plaster, as substrate, without prior inoculation had low growth ratings at the end of the 28-day test period. A comparison was made between plaster and wood specimens containing the same three coatings (Table 9). Fungal growth ratings for the two were nearly identical after both 7 and 14 days of exposure, but on extended exposure, painted wood, on average, supported slightly more growth than painted plaster.

Experiment #3. Effect of mixed fungal inocula and nutrient salts on the mildew susceptibility of three military specification paints on wood and steel.

In the third set of experiments, growth ratings were obtained from gumwood and steel specimens coated with non-fungicidal military paints. Steel panels, received painted, and wood specimens painted in the laboratory were inoculated with one of the following: ASTM fungal spore mixture, Method 508 salts, Method 508 fungal spore inoculum plus salts, or left unsprayed. Growth ratings obtained from exposure of these specimens in the cycled chamber are listed in Tables 10 and 11.

TABLE 7. Growth Ratings[a] for IBRG Panels

Substrate	Paint	Cycled Chamber Exposure in Days: 7	14	21	28
wood	A (linseed oil)				
	unweath control	1.5 (0.7)[b]	2.0 (0)	4.5 (0.7)	5.0 (0)
	inoc	3	3	5	5
	inoc + soil	3	3	5	5
	weath control	1.5 (0.7)	2.5 (0.7)	4.0 (0)	5.0 (0)
	inoc	2	2	4	5
	inoc + soil	3	4	5	5
wood	B (alkyd gloss)				
	unweath control	0 (0)	0 (0)	1.0 (0)	2.0 (0)
	inoc	2	2	3	4
	inoc + soil	3	4	5	5
	weath control	2.0 (0)	2.0 (0)	2.5 (0.7)	3.5 (0.7)
	inoc	3	4	5	5
	inoc + soil	3	4	4	4
wood	C (alkyd semi-gloss)				
	unweath control	1.0 (0)	1.0 (0)	2.5 (0.7)	3.5 (0.7)
	inoc	2	3	3	3
	inoc + soil	4	4	4	5
	weath control	0.5 (0.7)	1.5 (0.7)	2.5 (0.7)	3.5 (0.7)
	inoc	2	3	3	4
	inoc + soil	2	2	3	4
wood	E (PVA emulsion)				
	unweath control	2.5 (0.7)	3.5 (0.7)	4.5 (0.7)	4.5 (0.7)
	inoc	3	4	4	4
	inoc + soil	3	4	4	5
wood	F (alkyd mod. acrylic)				
	unweath control	1.0 (0)	2.5 (0.7)	4.0 (1.4)	4.5 (0.7)
	inoc	2	3	5	5
	inoc + soil	3	4	5	5
	weath control	0 (0)	1.5 (0.7)	3.5 (0.7)	4.5 (0.7)
	inoc	2	2	4	5
	inoc + soil	2	2	4	5

TABLE 7. Growth Ratings[a] for IBRG Panels (cont'd)

Substrate	Paint	Cycled Chamber Exposure in Days 7	14	21	28
wood	F_2(alkyd mod. acrylic + 1% TMTD)				
	unweath control	0 (0)	1.0 (1.4)	1.5 (0.7)	2.5 (0.7)
	inoc	2	2	2	2
	inoc + soil	3	3	3	3
	weath control	1.0 (0)	2.0 (0)	2.0 (0)	2.0 (0)
	inoc	3	3	3	3
	inoc + soil	3	4	4	5
wood	H (acrylic emulsion)				
	unweath control	3.0 (0)	4.0 (0)	4.5 (0.7)	5.0 (0)
	inoc	3	4	5	5
	inoc + soil	3	4	5	5
plaster	B (alkyd gloss)				
	unweath control	0 (0)	1.5 (0.7)	1.0 (0)	2.0 (0)
	inoc	2	2	2	3
	inoc + soil	4	5	5	5
plaster	F (alkyd mod. acrylic)				
	unweath control	0.5 (0.7)	0.5 (0.7)	0.5 (0.7)	0.5 (0.7)
	inoc	2	2	2	3
	inoc + soil	3	3	4	4
plaster	F_2 (alkyd mod. acrylic + 1% TMTD)				
	unweath control	1.0 (0)	1.5 (0.7)	1.5 (0.7)	1.5 (0.7)
	inoc	2	3	3	3
	inoc + soil	3	3	3	3

[a]Ratings represent single measurements except for controls which are the average of two measurements.
Rating scale as follows:

0 = no growth
1 = trace growth
2 = 1-10% coverage of test surface
3 = 10-30% coverage
4 - 30-70% coverage
5 = 70-100% coverage

[b]Standard Deviation

TABLE 8. Growth Ratings[a] for Weathered and Unweathered IBRG Wood Panels

	Cycled Chamber Exposure in Days			
unweath (5-coating control for weath)	7	14	21	28
no spray	0.7 (0.7)[b]	1.3 (1.1)	2.7 (1.6)	3.5 (1.3)
IBRG fungal inoculum	2.2 (0.4)	2.6 (0.5)	3.6 (1.3)	3.8 (1.3)
IBRG fungal inoculum + soil	3.2 (0.4)	3.6 (0.5)	4.4 (0.9)	4.6 (0.9)
weath (5 coatings)				
no spray	1.0 (0.8)	1.9 (0.6)	2.9 (0.9)	3.7 (1.2)
IBRG fungal inoculum	2.4 (0.5)	2.8 (0.8)	3.8 (0.8)	4.4 (0.9)
IBRG fungal inoculum + soil	2.6 (0.5)	3.2 (1.1)	4.0 (0.7)	4.6 (0.5)

[a]Rating scale as follows:

0 = no growth
1 = trace growth
2 = 1-10% coverage of test surface
3 = 10-30% coverage
4 = 30-70% coverage
5 = 70-100% coverage

[b]Standard Deviation

TABLE 9. Growth Ratings[a] for IBRG Wood and Plaster Panels Containing the Same Three Coatings

	Cycled Chamber Exposure in Days			
Wood	7	14	21	28
no spray	0.3 (0.5)[b]	1.2 (1.3)	2.2 (1.6)	3.0 (1.3)
IBRG fungal inoculum	2.0 (0)	2.3 (0.6)	3.3 (1.5)	3.7 (1.5)
IBRG fungal inoculum + soil	3.0 (0)	3.7 (0.6)	4.3 (1.2)	4.3 (1.2)
Plaster				
no spray	0.5 (0.5)	1.2 (0.8)	1.3 (0.5)	1.7 (0.5)
IBRG fungal inoculum	2.0 (0)	2.3 (0.6)	2.3 (0.6)	3.0 (0)
IBRG fungal inoculum + soil	3.3 (0.6)	3.7 (1.2)	4.0 (1.0)	4.0 (1.0)

[a]Rating scale as follows:

0 = no growth
1 = trace growth
2 = 1-10% coverage of test surface
3 = 10-30% coverage
4 = 30-70% coverage
5 = 70-100% coverage

[b]Standard Deviation

In the first experiment of this set with ASTM fungal spore mixture, no salts (Table 10), paints MIL-C-46168A and MIL-E-52835A supported growth on wood but not on steel. Even extending the cycled chamber exposure for 90 days did not produce growth on these coatings in this experiment. Paint MIL-E-52798A was judged to be the most microbially susceptible of the three coatings, as it was the only coating to support growth on both wood and steel. However, there is some inconsistency in the data from MIL-E-52798A because the 7- and 14-day ratings for steel panels were higher then ratings for comparable wood panels.

A second experiment with Method 508 fungal inoculum and salts (Table 11) was performed on similar steel specimens. In this case, all three military paints on steel supported growth within 90 days of cycled chamber exposure.

In general, MIL-E-52798A was again more susceptible to microbial growth than the other two paints. Steel, as a substrate, supported heavy growth only on the relatively susceptible MIL-E-52798A paint. Inoculation with salts alone had little impact on the growth ratings of the coatings, particularly for the two less susceptible coatings (MIL-C-46168A and MIL-E-52835A). Inoculation with fungal spore mixture plus salts resulted in increased fungal growth on coatings MIL-E-52835A and MIL-E-52798A at the start of the exposure. Despite the generally slower start for uninoculated coatings, each type of uninoculated paint coating supported growth at the 90 day termination of cycled chamber exposure.

Experiment #4. Effects of mixed fungal inocula and nutrient salts on mildew susceptible (no fungicide), marginally resistant (0.5% PMA-100), and resistant (2.0% PMA-100) vinyl acrylic paint coatings on wood.

Hardwood specimens were coated with defined vinyl acrylic paints and, after drying, inoculated with one of the following: IBRG fungal spore mixture, IBRG nutrient soil containing starch, IBRG fungal spore mixture plus nutrient soil, Method 508 fungal spore mixture in distilled water, Method 508 salts, Method 508 fungal spore mixture plus salts, or left unsprayed. After inoculation, the specimens were incubated in the cycled chamber. After six weeks of exposure the chamber experienced mechanical problems and was converted to uncycled operation to maintain sufficient ambient humidity. Growth ratings from the hardwood depressors are contained in Table 12.

The experiment on vinyl acrylic paint coatings in an extended exposure test was repeated to verify the effect of inoculation with and without salts on the microbial susceptibility of the coatings. Untreated coatings supported growth readily as contrasted to the two treated sets. Specimens coated with untreated paint and sprayed with Method 508 fungal spore mixture achieved high initial growth ratings. Method 508 salts per se made a negligible contribution to initial growth ratings. The data from the IBRG set were not consistent as nutrient soil alone increased the initial growth ratings of unprotected coatings, but in combination with IBRG fungal spore inoculum supplied no increase. After 14 to 21 days of incubation, all unprotected

TABLE 10. Growth Ratings[a] for ERADCOM Paints Sprayed with ASTM Fungal Inoculum

	Cycled Chamber Exposure in Days				
	7	14	21	28	90
MIL-C-46168A					
wood	3.3 (0.5)[b]	3.8 (1.0)	3.7 (0.8)	3.5 (1.0)	4.3 (0.5)
steel	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MIL-E-52835A					
wood	0.7 (0.8)	3.3 (0.8)	3.8 (0.4)	4.3 (0.5)	5.0 (0)
steel	0 (0)	-	0 (0)	0 (0)	0 (0)
MIL-E-52798A					
wood	0.8 (0.8)	3.0 (0)	4.2 (0.4)	4.3 (0.5)	5.0 (0)
steel	2.0 (0)	4.0 (0)	4.0 (0)	4.0 (0)	4.3 (0.6)

[a]Ratings represent the average of three specimens.

Rating scale as follows:

0 = no growth
1 = trace growth
2 = 1-10% coverage of test surface
3 = 10-30% coverage
4 = 30-70% coverage
5 = 70-100% coverage

[b]Standard Deviation

TABLE 11. Growth Ratings[a] for ERADCOM Paints on Steel Inoculated with Method 508 Fungal Spore Mixture and Nutrient Salts

	Cycled Chamber Exposure in Days				
	7	14	21	28	90
MIL-C-46168A					
no spray	0 (0)[b]	0 (0)	0.5 (0.7)	0 (0)	2.5 (0.7)
salts	0 (0)	0 (0)	0 (0)	0 (0)	3.3 (1.5)
inoc + salts	0 (0)	1.0 (1.0)	1.0 (1.0)	0.7 (1.2)	1.7 (1.2)
MIL-E-52835A					
no spray	0 (0)	0 (0)	0 (0)	0 (0)	1.5 (0.7)
salts	0 (0)	0 (0)	0 (0)	0 (0)	2.0 (1.0)
inoc + salts	2.7 (0.6)	1.3 (1.5)	1.0 (1.0)	0.7 (1.2)	2.0 (1.0)
MIL-E-52798A					
no spray	2.0 (0)	0.5 (0.7)	0.5 (0.7)	0 (0)	3.0 (1.4)
salts	1.5 (1.3)	1.3 (1.2)	1.7 (1.5)	1.3 (1.2)	2.7 (1.5)
inoc + salts	3.0 (2.6)	3.7 (2.3)	5.0 (0)	5.0 (0)	5.0 (0)

[a]Ratings represent the average of three specimens.

Rating scale as follows:

0 = no growth
1 = trace growth
2 = 1-10% coverage of test surface
3 = 10-30% coverage
4 = 30-70% coverage
5 = 70-100% coverage

[b]Standard Deviation

TABLE 12. Growth Ratings[a] for Hardwood Depressors Coated with Vinyl Acrylic Paints

	Chamber Exposure (Days)												
	Cycled				Uncycled								
	7	14	21	42	49	56	63	70	77	84	98	119	196
no PMA-100													
control	1.0(1.0)[b]	4.0(1.0)	4.3(0)	5.0(0)									
IBRG inoc	0 (0)	4.0(0)	4.7(0.6)	5.0(0)									
soil	2.0(1.0)	4.7(0.6)	5.0(0)										
inoc + soil	0 (0)	5.0(0)											
508 inoc	4.0(0)	5.0(0)											
salts	1.3(0.6)	3.7(0.6)	4.7(0.6)	5.0(0)									
inoc + salts	4.0(0)	5.0(0)											
0.5% PMA-100													
control	0 (0)	0 (0)	0 (0)	2.7(0.6)	3.7(0.6)	4.0(1.0)	4.0(1.0)	4.3(1.2)	4.3(1.2)	4.7(0.6)	4.7(0.6)	5.0(0)	
IBRG inoc	0 (0)	0 (0)	0 (0)	2.3(0.6)	4.0(0)	4.7(0.6)	4.7(0.6)	5.0(0)					
soil	0 (0)	0 (0)	0 (0)	2.0(0)	3.3(0.6)	4.0(0)	4.0(0)	4.3(0.6)	4.3(0.6)	5.0(0)			
inoc + soil	0 (0)	0 (0)	0 (0)	2.3(0.6)	3.7(0.6)	4.3(0.6)	5.0(0)						
508 inoc	0 (0)	0 (0)	0 (0)	2.3(1.2)	3.3(0.6)	4.3(0.6)	5.0(0)						
salts	0 (0)	0 (0)	0 (0)	3.0(1.0)	4.3(0.6)	4.7(0.6)	4.7(0.6)	4.7(0.6)	4.7(0.6)	4.7(0.6)	5.0(0)		
inoc + salts	0 (0)	0 (0)	0 (0)	3.0(0)	4.7(0.6)	5.0(0)							
2.0% PMA-100													
control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3(0.6)	1.0(1.0)	1.0(1.0)	1.0(1.0)	1.0(1.0)	4.3(0.6)
IBRG inoc	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7(0.6)	0.7(0.6)	1.0(0)	1.0(0)	4.7(0.6)
soil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.0(1.0)	1.3(0.6)	2.0(1.0)	2.0(1.0)	2.3(0.6)	2.3(0.6)	2.3(0.6)	4.0(1.0)
inoc + soil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3(0.6)	2.0(0)	1.7(0.6)	1.7(0.6)	1.7(0.6)	2.0(0)	2.0(0)	4.7(0.6)
508 inoc	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7(0.6)	1.3(0.6)	1.3(0.6)	1.3(0.6)	2.0(0)	2.0(0)	4.0(0)
salts	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3(0.6)	0.3(0.6)	0.3(0.6)	0.7(1.2)	0.7(1.2)	4.3(0.6)
inoc + salts	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3(0.6)	0.7(1.2)	1.0(1.0)	1.3(1.2)	1.7(1.5)	1.7(1.5)	5.0(0)

[a]Ratings represent the average of three specimens.

Rating scale as follows:
0 = no growth
1 = trace growth
2 = 1-10% coverage of test surface
3 = 10-30% coverage
4 = 30-70% coverage
5 = 70-100% coverage

[b]Standard Deviation

coatings achieved high growth ratings regardless of the presence or absence of the various pretreatments. Since chamber problems were experienced during this experiment, chamber conditions after 42 days may not have been optimal for the two treated sets of materials which supported growth late in the experiment. Coatings treated to be marginally resistant (0.5% PMA-100) supported growth at 42 days of incubation with negligible differences between categories of pretreatment, and after 56 days all categories achieved high growth ratings. Of the coatings treated to be fully resistant (2.0% PMA-100), specimens inoculated with IBRG nutrient soil, or IBRG soil plus IBRG fungal spore mixture, were first to support growth at 56 days of incubation, and all categories attained high growth ratings after 119 days of incubation.

DISCUSSION

Results obtained from exposure of paint coated specimens of varying fungal susceptibilities indicate that walk-in tropical chambers are suitable for the evaluation of paint films providing that test conditions are optimized to be conducive to fungal growth. Differences in results obtained in our chambers are attributed to differences in environmental conditions, substrate, fungal inoculum and nutrient salts or soil.

Good fungal growth was obtained on painted wood specimens exposed in the cycled tropical environment. This environment supplies a daily variation in temperature that is conducive to surface condensation. The importance of surface moisture for satisfactory growth has been discussed in the literature.[11,3] Since the cycled environment provides surface condensation, it is not surprising that the cycled chamber provided a better environment for fungal growth.

The ability of metal, as a substrate, to support less growth than wood is a familar observation.[12,3] Plaster and steel were more difficult to colonize as substrates than wood (Experiments #2 and #3). Coatings on wood panels supported more growth with increased chamber exposure than the same coatings on plaster. This difference can be attributed to extraneous nutrients supplied by the wood. The effect is least pronounced on panels treated with inoculum plus nutrient soil, presumably, because, in this case, the soil supplies sufficient nutrient to nearly mask the difference. Our work supports the findings of Zabel[13,14] that because wood *per se* is an exogenous carbon source, choice of substrate is an important parameter in determining the susceptibility of the coating. Wood as substrate without pretreatment supported the same amount of growth as comparable coatings on plaster pretreated with IBRG fungal inoculum but not quite as much as plaster pretreated with IBRG fungal inoculum plus IBRG nutrient soil containing starch (Experiment #2).

Inoculation with mixed fungal spores assured rapid growth, in the cycled chamber, on painted wood specimens. Paint effectively protected by PMA deterred growth of the fungal inoculum (Experiments #1 and #4). *Aureobasidium pullulans*, considered the most ubiquitous paint mildew organism, was included in two of the three fungal spore inocula. Although *Aureobasidium* was not included in Method

508.1 fungal spore inoculum, inoculation with Method 508.1 fungal spray resulted in satisfactory growth as compared with IBRG fungal inoculum in the only instance where a side-by-side comparison could be made (Experiment #4) -- possibly due to the presence of Aureobasidium in our tropical chamber environment. Aureobasidium will be included in a future revision of Method 508.

The use of a general purpose tropical chamber for screening purposes minimizes the question of which organism must be included for a test inoculum to be valid for a given test material. Since this type of chamber permits organism selection from an extensively diverse population, it can be argued that exposure in a general purpose chamber more closely approximates natural selection processes that occur during outdoor paint exposure tests and is therefore a more valid test environment than specialized cabinets containing only select organisms.

Another advantage of the tropical chamber is that fungal inoculum, though beneficial, is not strictly essential for the screening of susceptible coating-substrate pairings in a microbially active walk-in tropical chamber containing representative organisms common to nature. Our data indicate that uninoculated painted wood controls supported satisfactory growth within 21 to 28 days of incubation. Therefore, the fungal susceptibility of these coatings on wood would have been detected within the framework of a 28-day test. Also, since marginally protective treatments (Experiment #4) may require testing in excess of 28 days to determine relative effectiveness, an extended exposure test for 90 days may obviate the need to inoculate substrates more resistant than wood to encourage colonization during shorter test periods. For example, uninoculated coatings on steel supported satisfactory growth relative to inoculated coatings at 90 days of chamber exposure. However, growth ratings for less susceptible coatings (MIL-C-46168A and MIL-E-52835A) on steel even after extended exposure for 90 days were much lower than ratings obtained for the same coatings on wood. Inoculation with fungal spore spray was not sufficient to produce comparable growth for less susceptible coatings on steel. These results support Zabel's work in that choice of substrate is again shown to be an important parameter in determining the susceptibility of the coating (Experiment #3).[13,14]

Nutrient salts alone did not support improved growth response (Experiments #1, #3, and #4) presumably because trace mineral requirements of the fungi are already satisfied by nutrients from the paint, the substrate, or contaminants from the tropical chambers. The data suggest that, although nutrient salts may help to ensure a satisfactory fungus test, they are not strictly essential for the testing of paint coatings in a large tropical chamber.

The composite results from coatings on wood and plaster (Experiment #2) demonstrate that although inoculation with fungal spore mixtures increased initial growth on the coatings, most growth was obtained by pretreatment with IBRG fungal inoculum plus nutrient soil containing starch. This observation could not be strongly confirmed in data obtained from the last set of susceptible vinyl acrylic coatings on wood. Nevertheless, there were isolated instances where wood pretreated with nutrient soil supported more growth than would otherwise have been anticipated. The increased growth resulting from fungal inoculum plus soil can be attributed to both the physical action of the soil as a retention aid for the inoculum[3] and the presence of an exogenous carbon source.[3] In fact, Zabel and Terracina speculate that as preconditions for latex paint films to support microbial growth, the films must reach critical moisture levels and accumulate exogenous carbon sources.[13] If so, carbon sources should be available during the fungus test of latex films to simulate the effect of natural environmental contaminants accumulating during indoor and outdoor exposures. Our studies indicate that the differently pretreated coatings on wood supported nearly the same amounts of fungal growth near the end of the incubation period, so that for wood, as substrate, inclusion of an exogenous carbon source into the pretreatment was not essential. Painted plaster and steel were more difficult to colonize than wood substrates (Experiments #2 and #3). However, the same coatings on wood and plaster supported nearly the same amounts of fungal growth during the course of a 28-day test when both coatings were pretreated with IBRG fungal spore mixture plus nutrient soil. This observation supports the assertion of Bravery et al. that flat panels made from different substrates with appropriate pretreatment can produce comparable data.[3]

In our studies, inclusion of nutrients in the artificial soil provided not only an early stimulus to germination and growth but also instances of enhanced growth ratings relative to uninoculated coatings (Experiments #2 and #4). It appears that the inclusion of nutrients in the artificial soil can enhance the susceptibility of coating-substrate pairings, thereby producing comparable data from the same coatings on different substrates.

It cannot be determined from the limited scope of these studies whether the data obtained from inclusion or exclusion of nutrients in the artificial soil most closely reflects natural deterioration processes. However, availability of contaminants from various decaying materials in the tropical chamber would appear to lessen the necessity of adding nutrients in simulation of natural contamination of painted surfaces. The fact that coatings on plaster and steel without nutrient soil did support growth during tropical chamber exposure indicates that the tropical chamber met minimal fungal growth requirements without the necessity of added nutrients.

Artificial weathering increases the mildew susceptibility of paint coatings.[3,13] Weathering slightly increased the mildew susceptibility of both uninoculated and inoculated coatings on wood. Weathered coatings treated with IBRG inoculum plus nutrient soil were less susceptible than unweathered coatings through 21 days of exposure, but were equal at 28 days.

CONCLUSIONS

A cycled walk-in tropical chamber used for microbial screening of various Army materiel supported satisfactory fungal growth on flat painted surfaces of varying fungal susceptibilities. The ready availability, in this environment, of many representative organisms common in nature permits natural organism selection processes to occur in simulation of outdoor paint exposure.

Inoculation with fungal spore mixtures encouraged an early and uniform growth. Although prior inoculation was not strictly essential for the screening of susceptible coatings on wood in a microbially active tropical chamber, it was helpful for more resistant (or inert) substrates such as plaster and steel. Addition of nutrient soil containing starch to the inoculated surface enhanced growth attributable to both the presence of an exogenous carbon source and better retention of the inoculum. The enhanced growth resulted in comparable susceptibility of the same coatings on plaster and wood. Mineral salts per se assured that nutritional requirements were met but did not appear to substantially promote growth.

If tropical chamber testing is extended for 90 days to screen biocidal treatments, the need for prior inoculation of resistant substrates such as plaster and steel may be unnecessary. Uninoculated coatings on steel supported satisfactory growth relative to inoculated coatings after extended exposure for 90 days.

The data suggest that flat wood panels are a useful substrate, requiring no pretreatment with inoculum or nutrient soil, for the screening of paint films and biocides in a tropical chamber, and that other substrates, if pretreated with inoculum and nutrient soil, will give results more comparable to wood. Specialized test cabinets with specific test organisms do not appear to be strictly essential for evaluation of paint coatings.

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